MUMYCEL : Incidence of *Fusarium* mycotoxins multicontamination on human cells : cytotoxicity evaluation and toxicoproteomic approach.

Context and Problematic

Cereals are the most important resource for human and animal consumption in the world. According to forecasts by the Food and Agriculture Organization of the United Nations (FAO), the annual grain production is expected to increase to one billion tons, which is likely to cause problems of food security, especially on microbiological contamination, since the global use of pesticides has slowed considerably, forecasts confirm this trend. France is the first European producer and exporter of cereals. Cereal industry is an essential part of the economic fabric of the Brittany region (3.4 million tons of grain produced in 2010-2011, 5 million tons of processed cereals for animal nutrition and 38000 jobs - Passion Cereal, 2012). In cereals contaminant microorganisms, some species of fungi (*Aspergillus, Fusarium* and *Penicillium*) are toxigenic. In France, *Fusarium spp*. are the most problematic species because of their prevalence, ecology, physiology and wide range of mycotoxins (called Fusarium toxins) produced (Loos et al., 2004). Among *Fusarium*, three families are particularly important because of their high toxicity and their impact in European agricultural products: trichothecenes, fumonisins and zearalenone.

Chronic exposure to low doses of mycotoxins for long periods induces severe disease due to their power hepatotoxic, carcinogenic, neurotoxic ... (AFSSA, 2009). Severity of effects depends on the duration of exposure doses and combinations of toxins. *Fusarium species* can simultaneously producing different mycotoxins; moreover, matrices can be infected by several species of fungi. The co-presence of mycotoxins in cereals is a common situation. For example, 95% of corn harvested in France for the period 2004-2006, were multi-contaminated 3% were contaminated with trichothecenes and zearalenone, 30% by trichothecene and fumonisin and 65% by three fusariotoxins families (Arvalis, unpublished data). Presence of these multi- mycotoxin contaminations is a food safety and regulation problem. Food security, it is important to understand interaction between different mycotoxins to determine whether the effects are additive, synergistic or antagonistic. Concerning mycotoxin contamination regulation, it has been established considering mycotoxin individually. This is still the case in the European regulations (EU 1881/2006 Regulations). Lack of regulation on multicontaminations cereals and cereal products account is mainly due to the paucity of toxicological data and data on the overall effect of combinations of mycotoxins on cellular mechanisms.

In this project, we propose to better characterize impact of simultaneous presence of mycotoxins in acute and chronic mechanisms involved conditions (cytotoxicity, proteomics, transcriptomics) to have a better appreciation of risk "mycotoxins" in cereals. The prerequisite is to determine mycotoxins and mycotoxin associations to study. We focus our work on *Fusarium* studying mixtures of major mycotoxins. We will promote the association of mycotoxins produced by the same species of *Fusarium.*

	Type of cereals and Fusarium species							
	Wheat		Barley / Wheat				Maize	
	F. graminearum	*F. culmorum*	*F. poae*	*F. sporotrichoides*	*F. langsethiae*	*F. avenaceum*	*F. verticillioides*	*F. proliferatum*
Major mycotoxin								
Deoxynivalenol (type B trichotecene)	X	X						
Nivalenol (type B trichothecene)			X					
Diacetoxyscirpenol (type A trichothecene)			X					
T-2 toxin (type A trichotecene)			X	X	X			
Zearalenone	X	X						
Fumonisins							X	X

Table 1: Majors mycotoxines produced by different species of *Fusarium* (AFSSA, 2009)

Task 1 : Effect of chronic and acute exposition of mycotoxins combinations on Caco-2 and HepaRG cells : cytotoxic aspect

In the project, the toxicity of the mycotoxins coktails will be evaluated after chronic and acute exposition using *in vitro* cellular models. Although the cell lines behave differently than primary cultures, it doesn't always easy or possible to get primary cells. In this context, we propose to use the epithelial intestinal cell line Caco-2 since in the contaminated food, the intestinal epithelium constitutes the first defense barrier of the host and these cells are actively involved in the immune response (Oswald, 2006). We will also use the human hepatocyte cell line, hepaRG, since the liver represents the head of the metabolic conversion of food and toxic molecules.
It is well known that fumonisins and DON, the most prevalent trichotecen, modify the intestinal epithelial cells (Bouhet et al., 2006). Very few data exist about mycotoxins chronic expositions on Caco-2 (Van De Walle et al., 2010). Therefore, we will evaluate the effect of fusariotoxins, alone or in combinations, after a long-time exposition on Caco-2. Moreover, the human heptocytes hepaRG constitute a good model to study the hepatic metabolsim in toxicology (Guillouzo et al., 2007) and these cells will allow us to approach the real *in vivo* conditions.
In the task 1, two parameters will be followed : i) the cellular proliferation and the permeability of the cellular monolayer and ii) the secretion of proteins after acute and chronic expositions.

Task 2 : Effect of mycotoxins combinations on Caco-2 and HepaRG : molecular aspect

In parallel to toxicologic studies, proteomic and transcriptomic approaches will be used to elucidate the involved mechanisms and cellular pathways following an exposition to mycotoxins. The proteins involved in the response to the stress generated by the mycotoxins could be represent some targets of interest to elucidate the reactions cells/toxic molecules. The results should be provide new elements for the risks evaluation associated to the mycotoxins exposition in consumers. These functional analyses will be realized from samples of the two cell lines described above and incubated with different combinations of

mycotoxins (table 1) alone or in co-expositions, focusing the experimental conditions showing the most cytotoxicity. The concentrations of mycotoxins used in these molecular experiments will be subletal doses. The total intracellular proteins will be analysed by bidimensional electrophoresis (2D) coupled to tandem mass spectrometry. Following these experiments, potential biomarkers of exposition to mycotoxins, corresponding to the differential expressed proteins, would be identified.